Original Paper

Assessment of beta haemolytic streptococci carriage among school children in Calabar, Nigeria: A brief study

Mandor Baki Idasa 1, Lapah Pièrre Takem 2, Asuquo Anne E. 3, Ofonieme M. Ogba 3

1 Department of Microbiology and Parasitology, University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria.
2 Department of Pharmacology, Faculty of Basic Medical Sciences, University of Calabar, Cross River State, Nigeria.
3 Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, University of Calabar, Cross River State, Nigeria.

*Corresponding Author: Mandor Baki Idasa
Department of Microbiology and Parasitology, University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria.
E-mail address: kamdor1@yahoo.co.uk;

Running Title: Beta haemolytic streptococci Carriage among school children.

Received: 13 October, 2015; Revised: 17 December, 2015 Accepted: 25 December, 2015
Available online at http://www.thescientificpub.com http://dx.doi.org/10.19046/abp.v02i06.06

Abstract

Colonization of the upper respiratory tract of children by beta-haemolytic streptococci (BHS) is imperative in spreading the infection in households and in community settings. The present study was designed to investigate the prevalence of throat carriage of diverse antigenic groups of BHS and the influence of age on carrier rates in healthy children in Calabar. Throat swabs of 450 children’s were taken randomly from some government and private elementary schools and were visually examined and collected for further investigations. The swabs were cultured aerobically on goat blood agar. Antimicrobial sensitivity testing was carried out on all BHS isolates on Muller Hinton blood agar by using the Kirby-Bauer disc diffusion method after standardizing the inoculums with a 0.5 McFarland. A total of 18(4%) BHS pharyngeal carriers were detected. BHS belonging to four streptococcal groups were isolated, groups C, F and G. The fourth group was not identified by the available grouping kit (UIDS). Group C streptococci (GCS) had the highest percentage carriage of 5/18(27.8%). Other groups were F 3/18(16.7%), G 2/18(11.2%) and the UID 8/18(44.4%). Carrier rate increased as age group increased and peaked at age group 10-12 years 7(36.8%). The percentage of BHS pharyngeal carriage was higher in Government aided schools (6.7%) than in private schools (1.7%). The BHS were highly resistant to Penicillin and Ceftazidime with a resistivity rate of 94.7% while Augmentine showed the lowest resistivity rate of 15.8%. The study established a high carriage of diverse antigenic groups in the throat of children with age influencing the rate of carriage in this community. A throat carrier rate of 39% of GGS/GCS among 450 children from this population indicated the etiological importance of streptococcal pharyngitis in this population. Therefore, there is need for further studies to identify the specific serotype of the group C/G streptococci circulating in this community.

Keywords: Beta- haemolytic streptococci, Pharyngeal carriage, Group C streptococci, Group G streptococci.

Introduction

Carriage has been defined as the recovery of beta haemolytic streptococci (BHS) from the nasopharynx or oropharynx in the absence of any evidence of acute infection. Pharyngeal carriage of beta-haemolytic streptococci may lead to spread of respiratory infection in the community [1, 2]. Among the carriage groups, group A beta- haemolytic Streptococcus (GABS) is of specific importance and has remained a significant human pathogen for centuries. They produce a number of suppurative and non-suppurative infections in humans. Acute rheumatic fever (ARF) continues to be a leading cause of serious health problem in indigenous populations and developing
nations [3, 4]. These are the most common cause of pediatric heart disease worldwide with an annual incidence of 100-200 cases per 100,000 school aged children [5] and post streptococcal glomerulonephritis which may occur after throat or skin infections and certain brain disorders such as paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) are examples of non suppurative infections [6, 7]. BHS other than GAS (Groups G and C streptococci (GGS/GCS) have attracted attention in recent times as possible etiological agents of pharyngitis and post-streptococcal sequelae. Most importantly, species such as *Streptococcus dysgalactiae subsp. equisimilis* and *Streptococcus anginosus* belonging to group G and C respectively were commonly considered as throat commensals with a capacity to cause opportunistic infections in individuals with underlying medical conditions [8]. Recent reports by Reissmann et al [9] have however; shown these species emerging as an important cause of invasive disease and their large colony forming strains particularly resemble GAS in terms of virulence. There has been increasing reports on their association with streptococcal syndromes generally caused by GAS such as streptococcal toxic shock syndrome (STSS) [10] and acute rheumatic fever (ARF) [11]. This may be significant in countries where acute rheumatic fever continues to be a problem such as India. Children are the major reservoirs of GABHS and are most susceptible to its suppurative and nonsuppurative complications. Peak age of incidence of this infection is between 5-15 years and actually represents the pool from which adults with severe streptococcal invasive disease acquire their infections [2]. While these diseases (acute rheumatic fever and rheumatic heart disease) are in the decline in developed countries and continues to be a major cause of morbidity and mortality in developing countries of which Nigeria is no exception.

Because, colonization of the upper respiratory tract of children by BHS plays an important role in the spread of infection in the household and in community settings such as schools, day care centres and orphanages, it became imperative to initiate throat screening of healthy children to determine the prevalence of comparative throat carriage of diverse antigenic groups of BHS in a state like Calabar where no records of such information are found. This study is also aimed at determining the influence of age on the carrier rate of BHS in healthy children in this environ.

Materials and Methods

Subjects

A total of 450 apparently healthy children’s aged 3-15 years from different schools in Calabar, Nigeria were enrolled in this study after informed consent were obtained from their parents / guardians. Children were examined with regard to the presence of clinical symptoms such as sore throat, fever, chills, malaise and erythema, and swelling of the pharyngeal mucosa. Children who had any of these symptoms or had been on antibiotics for 2-5 days prior to the time of enrollment were excluded from the study.

Collection of specimen

The specimens were collected in various schools by staff of Microbiology Laboratory of the University of Calabar Teaching Hospital (UCTH) who were instructed to take the throat specimens with the aid of tongue depressor according to their routine method. Briefly, to rub a swab onto the tonsils and the soft palate. The study was approved by the ethics committee, UCTH.

Throat culture

All throat specimens were brought into diagnostic Microbiology Laboratory of UCTH in modified Stuart medium. The swabs were gently rolled over 1/3 of the surface of 5% goat blood agar plate, followed by streaking as described by Takem et al [12]. The plates were aerobically incubated at 37°C under 5% CO₂ for 24 h.

Confirmation of isolates

Colonies were identified as beta-haemolytic colonies based on their haemolytic pattern on blood agar, gram’s reaction and catalase reaction. Pure BHS colonies were stored in trypticase soya broth plus 20% glycerol and were immediately frozen at -20°C [13]. Bacitracin sensitivity testing was performed on all pure BHS colonies prior to storage. The purified isolates were serogrouped using the Strep PRO Grouping kit, (Hardy diagnostic, India).

Antimicrobial sensitivity assay

Antimicrobial disks were placed on the agar with sterile forceps. The agar plates were incubated inverted at 35°C for 18 hours. Zones of inhibition were measured and interpretation was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. The following antimicrobial agents were obtained as standard disc for their known potency in laboratory use, cefotaximes (Powercef (PB)), Graxone (GRA), Rocefin (R) 30ug, Ceftazidime (CAZ) 30 µg, Erythromycin (E) 15µg,
Penicillin G (P) 10 µg, Ampicillin (PN) 10 µg, Augmentin (AMC) 30 µg.

Statistical analysis
To compare the groups, the chi-square test with Yates’ correction was used; when the numbers were small Fisher’s exact test was used.

Results
A total of 18 BHS pharyngeal carriers were detected out of the 450 healthy children screened. BHS belonging to three main streptococcal groups were isolated C, F and G. Carrier females were 2.0 times more than carrier males. Carrier rate increased as age group increased and peaked at age group 10-12 years 7(36.8%), then it declined at age group 13-15 years. The most isolated streptococcal group was C with a percentage carriage of 27.8%, followed by group F(16.7%) and G(11.2%). Eight (44.4%) of the isolated BHS could not be identified by the antisera used and were name unidentified species (UIDs) as shown in Table 1. The percentage BHS pharyngeal carriage was higher in Government aided schools (6.7%) than in private schools (1.7%) (Fig 1).

Fig 2 shows the resistant pattern of the BHS isolated from the carriers with Penicillin and Cefazidime showing the highest resistivity rate of 94.7%, the resistivity rate of Ampicillin, Erythromycin, Grazone (Ceftriazone), Powercef (Ceftriazone), Rocephin (Ceftriazone), and Agmentine were 68.4%, 42.1%, 36.8%, 26.3%, 26.3% and 15.8% respectively.

Table 1: Distribution of carriers according to Age group, gender and streptococcal groups isolated.

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>Gender</th>
<th>Number (carrier rate)</th>
<th>BHS groups/percentage carriage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>C</td>
</tr>
<tr>
<td>3-6</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7-9</td>
<td>-</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>10-12</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>13-15</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 1: Distribution of carriers according to Age group, gender and streptococcal groups isolated.

Figure 1: BHS pharyngeal carriage in schools
PS=Private schools, GS=Government schools

Figure 2: Resistance pattern of BHS isolated from carrier
Discussion

The recent apparent resurgence of serious infections due to group A streptococci and their sequelae coupled with the attention attracted in recent times by non-GAS as possible etiologic agents of acute pharyngitis and post streptococcal sequelae calls for continuous surveillance of the carriage of beta-haemolytic streptococci is asymptomatic. Although many of such studies have been carried out all over the world, this study appears to be the first of its kind to be carried out in Calabar, Nigeria.

The study revealed an asymptomatic BHS pharyngeal carriage of 4.0% in which GCS had the highest carrier rate of 27.8% among the identified species. This is consistent with other studies worldwide which ranges from 2.5-35.5% although their subjects were of varying age [15-17]. The higher carrier rate of group C and G assumes importance clinically as they have been involved in human throat and other pyogenic infections as well as neonatal toxic shock syndrome, necrotizing fasciitis and other invasive infections elsewhere [18, 19]. Earlier studies however, have also shown GCS and GGS to be commonly isolated in temperate countries while group A are commonly isolated in the tropical countries [20].

The present study revealed no child at risk of developing RF or RHD as there was no GAS carriage during the study period and within the study population. This finding is consistent with unpublished reports in the pediatric department of the University of Calabar Teaching Hospital were cases of AGN are more frequently encountered in children in this environ than RF.

Several studies had shown carrier rate of beta-haemolytic streptococci to vary with age and season in healthy individuals. The peak observed in age group 10-12 years is similar to that observed by Kaplan et. al [21] although their study was based solely on GAS. The peak observed in this study could be attributed to the problem of teenagers making new friends in and outside their immediate environment where they may be exposed to new population of children harbouring different types of beta-haemolytic streptococci.

This study also revealed that more carriers were found in Government school (6.7%) children’s than private schools (1.7%). This is expected since these schools are Government aided schools (Universal Basic Education Scheme) and most of the children attending these schools belong to the lower socio-economic class and are more likely to live in poor housing conditions. Overcrowding in classrooms was a common observation in these schools with about 60-80 children per class. Aside being overpopulated, some of the classes did not have concrete floors and so were dusty, sanitary conditions were poor, and there was high traffic of pupils in and out of the classrooms raising dust particles. These conditions and others favour the spread of this infection. This finding is similar to that of Charmaine et al [7] where the conditions observed in the classrooms were the same though the schools were purposefully selected.

Although the treatment of non-GAS pharyngitis/tonsillitis has been a matter of controversy till date, some studies recommend that treatment may be clinically beneficial to the patient [22, 23] while others argue that the only benefit of treating such patients is for the relief of symptoms [15]. The recent number of biological and epidemiological lines of evidence suggesting that groups G and C streptococci might also cause rheumatic fever and glomerulonephritis [11], warranted the antibiogram testing. Nevertheless, the high resistivity of Penicillin and Ceftazidime found in this study could be attributed to the over use of these antibiotics in the treatment of this infection without proper laboratory diagnosis for fear of complications. Augmentin and the Ceftriazones showed low resistivity and so could be used as an alternative regime for treatment of the infection in this community.

Conclusion

A throat carrier rate of 39% of GGS/GCS among 450 BHS from this population indicated its possible importance in the etiology of streptococcal pharyngitis in this population. There is a need for further work to identify the specific M serotype of the group C/G streptococci circulating in this community. There may also be need to establish a link between the M serotype and the AGN cases more often encountered by clinicians in the community. The high carrier rate of the UIDs in this community calls for concern. In the event of their involvement in infections, treatment of such infections would be difficult.

Financial Assistance
None declared.

Conflict of Interest
The authors declare that there is no conflict of interest to reveal.
References


